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PII: S0141-8130(19)30235-1
DOI: https://doi.org/10.1016/j.ijbiomac.2019.05.009
Reference: BIOMAC 12294

To appear in: *International Journal of Biological Macromolecules*

Received date: 10 January 2019
Revised date: 30 April 2019
Accepted date: 3 May 2019

Please cite this article as: F. Chen, F. Xie, P. Liu, et al., Structure, thermal stability and suspension rheological properties of alcoholic–alkaline-treated waxy rice starch, International Journal of Biological Macromolecules, https://doi.org/10.1016/j.ijbiomac.2019.05.009

Structure, thermal stability and suspension rheological properties of alcoholic–alkaline-treated waxy rice starch

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Abstract:

In this work, the effect of alcoholic-alkaline treatment on the characteristics of waxy rice starch (WRS) and its aqueous suspensions was studied for the first time. Microscopy shows that the alcoholic-alkaline-treated WRS (AAT-WRS) was in the form of particles of lengths between 10 and 50 μm. Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) analyses indicate that the original A-type crystallites of WRS had been completely eliminated by the alcoholic-alkaline treatment and no new crystalline structure was formed. Fourier-transform infrared (FTIR) spectroscopy confirms the absence of chemical reactions during the treatment. Thermogravimetric analysis (TGA) results indicated the decreased thermal stability of AAT-WRS, which may be attributed to the amorphous structure and possible degradation resulting from the treatment. Compared with the gelatinized starch suspension, the AAT-WRS aqueous suspension presented a weaker shear-thinning behaviour, lower steady-shear viscosity, lower storage and loss moduli, and higher loss tangent, which suggest a less-rigid gel structure. The rigidity of the gel further decreased with higher temperatures. Our results here could provide insights into the rational design of modified starch products with waxy rice starch.

Keywords: waxy rice starch; alcoholic-alkaline treatment; morphology; crystallinity; thermal stability; rheological properties

1 Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops and is a staple food source for half of the world's population [1]. The major component of rice is starch, which affects the processability of rice [2]. Starch contains two types of biomacromolecules, namely amylose and amylopectin. Amylose is a sparsely branched carbohydrate mainly based on α(1–4) linkages with a molecular weight of 10^5–10^6 and have a degree of polymerisation (DP) as high as 600 [3]. Amylopectin is also based on α(1–4) linkages (about 95%) but is a multiple-branched polymer via α-(1,6) linkages (about 5%), with a high molecular weight of 10^7–10^9 [3]. Normal and waxy rice cultivars are generally classified according to their amylose contents in the starch. Normal rice starch is composed of 20–30% linear amylose and 70–80% highly branched amylopectin, while waxy starch consists of almost amylopectin. The amylose content determines rice starch characteristics [4].

In order to modify the functional properties of starches and to expand their applications, various physical and chemical modification methods have been developed [5]. Granular cold-water-soluble (GCWS) starches are an important group of starch product that can hydrate rapidly without heating to produce the functionalities of cook-up starches [6]. This type of starch has high potentials not only in food applications but also in other areas such as encapsulation and controlled release [7-11], consolidation of porous mullite ceramics [12], and sizing of textiles [13].

Different methods have been developed to prepare GCWS starches [6]. Eastman and Moore [14] published a patent which describes the preparation of a GCWS starch by heating ungelatinized maize starch in 75–90% ethanol to 150–175 °C for 0.5–2.0 h. This type of GCWS starch was suggested to exhibit a V-type (single-helix) crystalline structure, which is likely to be converted from native

amylopectin double helices [15, 16]. Later, Rajagopalan and Seib [17-19] introduced a modified method that uses a polyhydric alcohol, allowing the heating at atmospheric pressure. Dries et al. [20] prepared a GCWS maize starch by an aqueous ethanol treatment at elevated temperatures and they found that the amylose fraction contributes almost exclusively to the V_H-type crystal formation in the product.

Jane et al. [21-23] introduced a different, simpler method to prepare GCWS starches using an alcoholic-alkaline treatment at ambient temperature followed by oven drying. This method has been shown to be effective for various starches such as maize [10, 11, 22-31], potato [11, 24, 32, 33], buckwheat [34], banana [35], and chickpea [36]. Most GCWS starches showed reduced to non-crystallinity [10, 13, 24, 32-34, 36]. In this method, sodium hydroxide breaks the intermolecular hydrogen bonds in native starch granules, while ethanol inhibits the swelling of the granules and maintains their integrity. When ethanol is evaporated, a cavity is formed in the hilum of starch granule and brings about a metastable starch granule with excellent cold water swellability [26]. The treated GCWS starches showed V-type patterns for normal and high-amylose maize starches [10, 23, 31] and buckwheat starch [34], whereas the GCWS waxy maize starch was amorphous [23]. The crystalline pattern of a GCWS potato starch was reported to be either the B-type or [32] or V-type [33]. The suspension viscosity of GCWS starches was observed to be either increased [23, 27, 35] or decreased [25, 33, 34], which may depend on the type of starch and treatment conditions. For maize starches with different amylose contents, a 70–90% cold-water solubility was achieved [23]; treatments at a lesser concentration of ethanol, a greater concentration of NaOH, and a higher reaction temperature produced GCWS starches with a greater cold-water solubility [22]. A GCWS

potato starch showed a higher cold water solubility than a GCWS maize starch that was similarly prepared [24]. Further studies [29, 31] have shown that the ultrasound-assisted alcoholic-alkaline treatment could greatly improve the solubility of GCWS maize starch with solubility up to 93.87%. Moreover, compared with oven-drying after the alcoholic-alkaline treatment, freeze-drying was shown to result in a GCWS maize starch with a higher solubility of 85.3% [30]. So far, the structure and properties of rice starch, especially waxy rice starch, subject to alcoholic-alkaline treatments have not been studied.

The purpose of the present study was to investigate the morphology, structure, thermal stability, and suspension rheological properties of WRS treated using an alcoholic-alkaline method. Due to the lack of amylose, we found that WRS presents particular changes to, for example, the granule morphology (agglomerated granules without wrinkled or indented morphologies) and the crystalline structure (lack of V-type crystallites) compared with other types of starch after the alcoholic-alkaline treatment. Our results here are expected to add new knowledge to the field of physical modification of starch.

2 Materials and methods

2.1 Materials

Waxy rice was purchased by COFCO Haiyou (Beijing) Co., Ltd. (Beijing, China), which has a brand name of Youcai Wuchang Glutinous Rice and is originated from Wuchang, Heilongjiang, China. Sodium hydroxide was supplied by Fuchen (Tianjin) Chemical Reagents Co., Ltd. (Tianjin,

China). Absolute ethanol was supplied by Tianjin Fuyu Fine Chemicals Co., Ltd. (Tianjin, China). Hydrochloric acid was supplied by Guangdong Guangshi Reagents Technology Co., Ltd. All these chemicals were of analytical grade. Double-distilled water was used in all experiments.

2.2 Sample preparation

2.2.1 Isolation of waxy rice starch (WRS)

The waxy rice was crushed into powder using a Baijie multifunctional crusher (BJ-150, Deqing Baijie Electric Appliances Co., Ltd., Deqing, Zhejiang, China), which was passed through a 100-mesh sieve. Starch was extracted by an alkaline steeping method [37]. 100 g (dry basis) of waxy rice flour were soaked with 500 mL of 0.2% (w/w) NaOH solution for 24 h. The supernatant was drained off and the sediment was washed to remove the protein using 0.2% (w/w) NaOH until the yellow layer disappeared. The starch residual was then washed with distilled water, neutralized by 1M HCl, washed with distilled water, and centrifuged (TDL-5-A, Shanghai Anting Scientific Instrument Factory, Shanghai, China) at 5,000 rpm for 10 min. After dying at 50 °C for 48 h, the starch was passed through a 100μm sieve and stored in a desiccator. The crude fat, protein, and ash contents of the isolated waxy rice starch (WRS) were analysed before further use.

2.2.2 Preparation of alcoholic-alkaline-treated waxy rice starch (AAT-WRS)

The preparation of alcoholic-alkaline-treated waxy rice starch (AAT-WRS) followed the method of Chen & Jane [22] with slight modification. Native WRS (10 g, dry base) was suspended in 70 g absolute ethanol in a 250mL beaker equipped with a magnetic stirrer (JB300-SH, Shanghai Specimen and Model Factory, Shanghai, China) and the temperature of the suspension was

maintained at 25 °C. The suspension was first slowly added with 19 g of 3M NaOH solution and then with 20 g of absolute ethanol, followed by additional 9 g of 3M NaOH solution. The slurry was allowed to stand for 25 min with gentle stirring. The supernatant was removed and the collected starch was washed with 80% (w/w) ethanol solution. After being resuspended in sufficient 80%, (w/w) ethanol solution and neutralized with HCl (3 M in absolute ethanol), the sample was washed with 95% (w/w) ethanol solution and dehydrated with absolute ethanol. The obtained AAT-WRS was oven-dried at 50 °C for 12 h. The dried starch was ground and passed through a screen to give an average particle size of 100 μm and stored at room temperature before analyses.

2.3 Characterization

2.3.1 Chemical analysis

The general properties of native WRS as the control were analysed using the AOAC method. Water content was obtained by oven drying using a blast-drying oven (101-2A, Tianjin Taisite Instruments Co., Ltd.) at 105 °C for 6 h. Crude fat content was measured using a Soxhlet extraction fat analyser (SZF-06A, Shanghai Jingqi Instruments Co., Ltd., Shanghai, China). Crude protein content was measured using an automatic Kjeldahl apparatus (KDN-103F, Shanghai Xianjian Instruments Co., Ltd., Shanghai, China). Crude ash content was measured in a crucible (550–600 °C) using an oven (SXL-1208, Shanghai Jinghong Experimental Instruments Co., Ltd., Shanghai China). All measurements were repeated thrice and the mean values were obtained.

2.3.2 Light microscopy

Light micrographs of native WRS and AAT-WRS were taken in deionized water or ethanol with a polarization microscope (Axioskop 40 Pol/40 A Pol, Zeiss) equipped with a 35mm SLA camera. The magnification was set at 400. Both ordinary and polarized lights were used to investigate the morphologies of starch samples.

2.3.3 Scanning electron microscopy (SEM)

Starch samples were sprinkled on double-adhesive tape fixed on aluminium stubs, which were then coated with gold/palladium for 2 cycles of 40 s/mA using a sputter coater (208HR, Cressington Scientific Instruments Ltd., Watford WD19 4BX, England, UK). Scanning electron micrographs of native WRS and AAT-WRS starches were acquired with a ZEISS SIGMA field-emission scanning electron microscope (Carl Zeiss AG, Oberkochen, Germany) with an InLens detector, an electron high tension (EHT) voltage of 6 kV, and an aperture of 20 µm, operated by ZEISS SmartSEM software (v. 6.03).

2.3.4 Differential scanning calorimetry (DSC)

Thermal properties of native WRS and AAT-WRS were analysed using a PerkinElmer DSC4000 facility (PerkinElmer, Inc., Waltham, Massachusetts, USA) equipped with a thermal analysis data station. 3.5 mg (dry basis) of starch was weighed into a 40µL capacity aluminium pan and distilled water was added to achieve a starch suspension containing 70% as water. The samples were hermetically sealed and allowed to stand for 1 h at room temperature before DSC measurements. The DSC analyser was calibrated using indium and an empty aluminium pan was

used as the reference. Starch samples were heated at a rate of 10 °C/min from 20 to 100 °C under nitrogen. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy of gelatinization (ΔH) were calculated.

2.3.5 Powder X-ray diffraction (pXRD)

Powder X-ray diffraction (pXRD) patterns of starch samples were acquired using a PANalytical Empyrean X-ray diffractometer (Malvern Panalytical Ltd, Malvern, UK) equipped with a reflection-transmission spinner as the sample stage, a Co target with an intended wavelength to be Kα = 1.790307 Å, and a PIXcel1D (RTMS type) detector. Starch samples to be tested were compressed in a designated powder sample holder for the diffractometer. pXRD patterns were recorded for an angular range (2θ) of 6−40°, with a step size of 0.0263°, a total of 1295 points, and total scan time for about 46 min. The radiation parameters were set at 40 kV and 40 mA, with a fixed incident beam mask of 10 mm, a Si offcut, and a beam knife.

2.3.6 Attenuated total reflectance Fourier-transform infrared (ATR-FTIR)

ATR-FTIR spectra of different starch samples were recorded using a Bruker TENSOR 27 FTIR spectrometer (Bruker Corporation, Billerica, Massachusetts, USA). For each spectrum, 32 scans were recorded over the range of 4000−500 cm^{-1} at room temperature (about 22 °C) at a resolution of 4 cm^{-1}, co-added and Fourier-transformed. The background spectrum was recorded on air and subtracted from the sample spectrum.

2.3.7 Thermogravimetric analysis (TGA)

A Mettler Toledo TGA facility (Mettler Toledo, Columbus, Ohio, USA) was used with 70μL alumina crucibles for thermogravimetric analysis (TGA) under nitrogen. A sample mass of about 3 mg was used for each run. The samples were heated from 25 to 700 °C using a constant heating ramp of 10 K/min.

2.3.8 Cold water solubility

Cold-water solubility was determined using the method by Eastman and Moore [14] with slight modifications. AAT-WRS suspensions (1%, w/w) were prepared and stirred at 100 rpm with a magnetic stirrer (JB-3, Changzhou Aohua Instruments Co., Ltd., Shanghai, China) for 30 min at ambient temperature (25 °C), then centrifuged (TDL-5-A, Shanghai Anting Scientific Instrument Factory, Shanghai, China) at 3500 g for 10 min. The supernatant was poured into a pre-weighed plate and dried at 105 °C for 4 h, then immediately weighed the dissolved solids. The cold-water solubility was calculated by the following Eq. (1):

$$\%\ \text{water solubles} = \frac{\text{weight of solids in supernatant}}{\text{weight of original solids}} \times 100 \quad (1)$$

2.3.9 Rheological properties

For preparing a native WRS paste, certain amounts of native WRS (dry basis) and water were mixed at a concentration of 5% (w/w). The suspension was heated at 95 °C using a water bath for

30 min to form a paste. To obtain an AAT-WRS paste, AAT-WRS was mixed with water at room temperature at a concentration of 5% (w/w).

The rheological properties of starch pastes were tested using a TA Discovery HR-2 rheometer (New Castle, DE, USA) with a 40mm diameter parallel plate geometry and a Peltier plate temperature system. The distance between the plates was set at 1 mm. After loading the sample between the plates, the excess sample outside the plates was removed. Silicone oil (Shenzhen Jipeng Silicon Fluorine Material Co., Ltd.) was placed around the edge of the measuring cell to prevent the changes in moisture contents during measurements from the environment. Silicone oil would hardly affect the experimental results as it is immiscible with polysaccharide solutions and has a relatively lower viscosity [38, 39].

Steady-state viscosities of starch samples were recorded at shear rates from 0.1–500 s^{-1} at a fixed temperature of 25 °C.

Dynamic rheological properties such as storage modulus (G'), loss modulus (G''), and loss factor (tan δ) were determined for starch samples as well. An angular frequency sweep from 0.1–20 rad/s was undertaken with a fixed temperature of 25 °C and a fixed strain of 1.5%. A temperature sweep from 25–100 °C at 5 °C/min was undertaken with a fixed angular frequency of 5 rad/s and a fixed strain of 1.5%. The fixed strain of 1.5% was chosen based on our preliminary experiments to determine the fluid's linear viscoelastic region.

3 Results and Discussion

3.1 Chemical analysis

Chemical analysis indicated that AAT-WRS had a moisture content of 7.34±0.03%, protein content of 0.16±0.01%, fat content of 0.26±0.00%, and an ash content of 0.16±0.01% (average ± standard deviation). These data indicate a high purity of native WRS after extraction from waxy rice starch.

3.2 Morphology

Fig. 1 shows the light microscopic images of native WRS and AAT-WRS. Under normal light, native WRS is observed to be in small particles with polyhedral shapes and varied particle sizes [40]. Birefringence patterns (Maltese crosses) can be clearly seen for native WRS granules under polarized light, indicating the original spherulites and crystalline structure of starch. After alcoholic-alkaline treatment, AAT-WRS presented intact granules as well as clumps, which can be seen as agglomerations of granules. Maltese crosses disappeared completely, showing the crystalline structure has been predominantly destroyed by the alcoholic-alkaline treatment, which is in agreement with previous studies on different maize starches [10, 23] and potato starch [33]. However, both native and GCWS buckwheat starches were reported to present similar birefringence under polarized light [34].

Fig. 2 shows the SEM images of native WRS and AAT-WRS. It can be clearly seen that native WRS has polyhedral shapes with smooth surface and diameters ranging from about 3–7 μm. For

AAT-WRS granules, a very different morphology was shown. It seemed that starch granules had fused together to form clumps of lengths between 10 and 50 μm. The agglomeration of treated GCWS starch granules has also been observed for maize starches with different amylose contents [10, 11]. The surfaces of the clumps were smooth and stuck with some small particles. Some holes and crevices are seen on the clumps, which could be due to the spaces between agglomerated granules. Wrinkled or indented morphologies, which were previously reported for other types of starch (e.g., regular and high-amylose maize starches [10, 22, 25, 27, 31], potato [24], and chickpea starch [36]) and could be ascribed to the leaching of amylose molecules from granules [22, 41], were not evident here for AAT-WRS. This is as expected as WRS used here contains mostly amylopectin.

3.3 Crystallinity

Fig. 3 shows the DSC heat flow curves for native WRS and AAT-WRS. Native WRS displayed a distinct thermal transition, which could be undoubtedly ascribed to the gelatinization of starch. Based on the curve, thermal parameters can be calculated: T_o = 62.5 °C, T_p = 70.4 °C, T_c = 82.1 °C, and ΔH = 11.9 °J/g. In contrast, no transition could be observed for AAT-WRS, suggesting the alcoholic-alkaline treatment had totally destroyed the crystalline structure of native starch. Similarly, previous research showed that no gelatinization peak could be observed for GCWS starches derived from potato [33] and regular maize [13, 23], and high-amylose maize [23]. In some other studies, GCWS starches displayed significantly reduced (but not zero) enthalpies compared to their native counterparts such as buckwheat [34] and chickpea [36].

Fig. 4 shows the XRD patterns of native WRS and AAT-WRS. Native WRS showed strong reflections at 2θ of about 17.5° (d-spacing = 5.88 Å) and 26.8° (3.86 Å) and an unresolved doublet at 2θ of 19.8° (5.21 Å) and 20.8° (4.96 Å), with a few weak peaks at 2θ of about 11.6 (8.86 Å), 13.0 (7.91 Å), 23.1° (4.47 Å), 30.4° (3.41 Å), and 35.4° (2.94 Å). Similar XRD patterns measured using the same ray source (Co Kα) were shown for starch A-type crystallites [42, 43]. Our XRD pattern shown here is also similar to those observed for waxy rice starches elsewhere [1, 44] although there are minor differences in peak location due to the different X-ray sources used. These reflections should be considered as a typical A-type pattern as reported previously [40, 45-47], although the previously reported peak at 2θ = 15 was not evident here. In contrast, AAT-WRS was observed to be completely amorphous, with the disappearance of all these diffraction reflections. This result well corresponds to the DSC and microscopic analyses, which shows that the alcoholic-alkaline treatment could lead to a complete loss of the original A-type crystalline structure. In previous research, GCWS starches also showed significantly reduced, or a complete loss of, crystallinity according to XRD for high-amylose maize [10], regular maize [31], potato [33], and buckwheat [34] starches. GCWS potato starches showed the B-type pattern in XRD with crystallinity reduced from 35% to 28% [32]. According to Chen and Jane [23], a strong alkaline solution could dissociate protons of the ─OH group and make them negatively charged, resulting in swelling of starch granules. The swelling of the granules could break up starch crystallites and lead to uncoiling or dissociation of double helices. Moreover, after alcoholic-alkaline treatments, a V-type XRD pattern was evident for high-amylose maize starches [10, 11, 23], regular maize starch [11, 31], potato starch [33], buckwheat starch [34]. Expectedly, the V-type pattern was not observed here for AAT-WRS as a

waxy starch containing mostly amylopectin was used here [11, 23]. This is despite that long branch chains of amylopectin were also suggested to contribute to the content of V-type crystalline structure during ethanol precipitation under pressure [16].

3.4 FTIR

Fig. 5 shows the FTIR spectra for native WRS and AAT-WRS. For native WRS, there are a few characteristic bands of starch. These peaks below 900 cm^{-1} (the fingerprint region) with major bands at 851 cm^{-1}, 762 cm^{-1}, and 706 cm^{-1} can be attributed to the skeletal modes of the pyranose ring in the glucose unit of starch [48, 49]. The peaks at 927 cm^{-1} and 851 cm^{-1} can be attributed to the presence of C─OH bonding, the vibration of C─O─H deformation and CH_2-related modes [48, 49]. Peaks at 1078 and 997 cm^{-1} are characteristic of the C─O bond stretching of the anhydroglucose ring [50]. The band at 1339 cm^{-1} can be ascribed to O─C─H, C─C─H and C─O─H while the band at 1150 cm^{-1} should be attributed to the coupling of C─O, O─H and C─C bonds. A characteristic peak occurred at 1641 cm^{-1}, which is presumably a feature of tightly bound water in starch [50], namely H─O─H bending vibrations that indicate inter- and intramolecular hydrogen bonding [48, 49]. The band at 2932 cm^{-1} is characteristic of the C─H stretching of the glucose unit [48-50]. An extremely broad band due to hydrogen-bonded hydroxyl groups (O─H) appeared at 3310 cm^{-1} which can be attributed to the complex vibrational stretches associated with free, inter- and intramolecularly bound hydroxyl groups which make up the gross structure of starch [50, 51]. For AAT-WRS, the positions of these bands do not change obviously, which means the alcoholic-alkaline treatment did not result in any chemical reactions [13, 31].

3.5 TGA

Fig. 6 shows the thermal stability of native WRS and AAT-WRS as detected by TGA with a temperature scan up to 700 °C. It can be seen that native WRS displayed a small derivative peak up to about 110 °C, which could be ascribed to the evaporation of moisture. After that, a major weight loss observed as two overlapped peaks occurred from about 260 to 530 °C, which could be ascribed to the thermal decomposition of starch. This is somewhat different to the TGA results for maize starches, which have shown that the major thermal decomposition, which could be associated with the breakage of long chains of starch as well as the destruction (oxidation) of the glucose rings, takes place between 240–330 °C [52, 53]. On the other hand, AAT-WRS exhibited a similar weight loss of moisture before 110 °C but a very different profile for the starch thermal decomposition. The associated two peaks moved to low temperatures. Regarding this, the alcoholic-alkaline treatment could destroy the crystalline structure and possibly degraded the starch molecules, resulting in reduced thermal stability.

3.6 Cold water solubility

Table 1 shows the cold-water solubility data of native WRS and AAT-WRS. The solubility of normal WRS was low (4.40%), whereas a significantly increased cold-water solubility was obtained for AAT-WRS (68.13±0.96). This was also observed previously for various starches such as waxy maize [22], regular maize [13, 24, 25, 27, 29-31], high-amylose maize [22], potato [24, 33], buckwheat [34], and banana [35]. For example, according to Chen and Jane [23], GCWS maize starches showed fully swollen granules when dispersed in cold water and exhibited a 70–90% cold-

water solubility. Yu et al. [31] demonstrated that an ultrasound-assisted alcoholic-alkaline treatment could increase the solubility of AAT-WRS from 69.24% to 75.05%, whereas the solubility of native maize starch was only 4.18%. It has been suggested that alkali conditions can break the intermolecular hydrogen bonds, therefore enhancing the water solubility [54]. However, alcohol can restrict the swelling of granules and retard the dissociation of the native, double-helical structure [22]. Therefore, suitable contents are alkaline and alcohol are important for creating GCWS starches with high solubilities. Here, the tremendously increased solubility of GCWS starches could also due to the loss of crystallinity [33]. Moreover, solubility was found to be greatly influenced by temperature [13, 22, 32, 34]. For example, Zhu et al. [13] reported the solubilities at 25 °C and 35 °C of oven-dried GCWS regular maize starch were 46.33% and 70.48%, respectively; and those at 25 °C and 35 °C of the freeze-dried GCWS sample were 55.71% and 89.62%.

3.7 Rheological properties

Fig. 7 shows the steady shear viscosity changes as a function of shear rate for native WRS and AAT-WRS. The relationship between viscosity and shear rate results can be fitted using the classic power-law equation [39, 55, 56]:

$$\eta = K\dot{\gamma}^{n-1} \quad (2)$$

In this equation, η is viscosity, $\dot{\gamma}$ is shear rate, n is power-law index, and K is consistency (which is the viscosity at 1 s^{-1} shear rate). A greater slope of the viscosity–shear rate curve indicates a stronger

shear-thinning behaviour. It can be seen that for native WRS in water without heating to gelatinize the starch granules, the viscosity was very low and did not change with shear rate, indicating that the suspension was mostly a Newtonian fluid, similar to water. Correspondingly, *K* was very small and *n* was very close to 1. After gelatinization, native WRS displayed significantly increased viscosity (*K* = 2.556) and a strong shear-thinning behaviour (*n* = 0.983). This is as expected as gelatinization is a process that is featured by the absorption of large amounts of water by the granules and the diffusion of starch molecules into water, which leads to the formation of a viscous, shear-thinning paste [57]. In comparison, the AAT-WRS suspension displayed a lower viscosity than that of the native WRS suspension. In addition, the slope of the fitted viscosity–shear rate curve was less steep for AAT-WRS than for native WRS. Along with those, AAT-WRS had *K* of 0.532 and *n* of 0.687. These results show that the AAT-WRS suspension was less viscous and had a less shear-thinning behaviour compared to the gelatinized native WRS suspension. Similarly, RVA results at room temperature (25 °C) showed that for buckwheat starches [34], GCWS starch suspensions had reduced viscosities than the native counterpart suspensions. Moreover, using a typical RVA profile with heating, the viscosities of GCWS potato [33] and buckwheat [34] starch suspensions did not show a gelatinization peak, which means the alkaline treatment had completely destroyed the original crystalline structure. Moreover, Chen and Jane [23] reported that GCWS waxy and high-amylose maize starches exhibited viscosities higher than those of their native counterparts, but the viscosity of the treated regular maize starch was lower than that of its native starch. Bello-Pérez et al. [35] also reported reduced viscosity and shear-thinning behaviour for GCWS regular maize starch where GCWS banana starch suspensions displayed higher viscosities and stronger shear-thinning

behaviours than the native counterpart suspension. It is likely that the molecular structure of starch could play an important role in determining the rheological behaviour of GCWS starches.

The dynamic viscoelasticity of native WRS and AAT-WRS was also studied and the results are shown in Fig. 8. Frequency sweep tests (Fig. 8a) indicate that, for the gelatinized native WRS suspension, both G' and G'' increased with increasing frequency and G' was also higher than G'' while the corresponding tan δ did not experience significant changes. Similar results were also observed for starch/polysaccharide pastes and gels [58]. This means that the gelatinized native WRS suspension was more elastic than being viscous. It has been suggested that starch dispersion can be described as a composite and its viscoelastic properties in the pasted and in the gelled state are primarily governed by the volume fraction occupied by the swollen granules as a dispersed phase, which accounts for two-thirds of the total volume [58]. For the AAT-WRS suspension, higher frequencies led to more significant increases in both G' and G'' while tan δ experienced a decrease, A similar trend was observed before for GCWS potato and maize starches [24]. Compared with the native WRS suspension, the AAT-WRS suspension presented lower G' and G'' and higher tan δ values, indicating that the treated-starch suspension had a less-rigid gel structure. Regarding this, it was likely that the AAT-WRS system was more homogenous due to the dissolution of the treated starch; as a result, the viscoelasticity of such a system was more dominated by a continuous phase of the dissolved macromolecules.

Fig. 8b shows that, with increased temperature, G', G'', and tan δ all experienced slight decreases for the gelatinized native WRS suspension. This could be expected as after gelatinization a network structure had already been formed and only the molecular mobility could be increased by higher

temperature. For the AAT-WRS suspension, increasing the temperature were seen to lead to more apparently reduced G' and G'' up to about 85 °C. Previous studies have shown either increased or decreased G' and G'' for GCWS starches depending on the type of starch [24, 33]. Regarding our results, it could be speculated that higher temperatures could allow greater starch molecular mobility and starch–water interactions for AAT-WRS, which enhanced the solvation effect and reduced the rigidity of the gel structure. At even higher temperatures, both G' and G'' increased. While no significant changes were observed for tan δ before about 75 °C, after that, a decrease in tan δ was evident. Given this, when the temperature was high enough, it was likely that the starch chain interactions could be enhanced, leading to a more rigid gel structure.

4 Conclusions

Using an alcoholic-alkaline treatment, we prepared AAT-WRS with a starch extracted from a waxy rice cultivar originating in northeast China, which showed a solubility of 68.13%. The significantly increased solubility could be ascribed to the complete amorphous structure resulting from the alcoholic-alkaline treatment as shown by DSC and XRD. FTIR spectroscopy shows that native WRS and AAT-WRS are identical in chemistry, therefore, confirming simply a physical process occurring to WRS. Nevertheless, this treatment led to the reduced thermal stability of AAT-WRS. Dissolving AAT-WRS in water could result in a fluid with a less rigid gel structure and less shear thinning behaviour compared for the typical gelatinized starch suspension. Increasing temperature might allow greater starch–water interactions, which could further reduce the rigidity of

the system. Our results here could provide new understandings for further design of modified starch products.

Acknowledgements

This research was funded by the China Postdoctoral Science Foundation (grant No. 2017M612679), the Sailing Scheme of Guangdong Province (grant No. 2017YT05H077), the Pearl River S&T Nova Program of Guangzhou (grant No. 201610010019), the Guangxi Key Laboratory of Polysaccharide Materials and Modification, Guangxi University for Nationalities (grant No. GXPSMM18ZD-02), and the Natural Science Foundation of Guangdong Province (project No. 2018A0303130048). F. Xie acknowledges the support from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 798225.

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Tables

Table 1. Solubilities of native WRS and AAT-WRS in water at ambient temperature (25 °C).

Sample	Solubility in water (%)
Native WRS	4.40±0.01
AAT-WRS	68.13±0.96

Figure Captions

Fig. 1. Light microscopic images of native WRS in water and AAT-WRS in ethanol under normal and polarized lights.

Fig. 2. SEM images of native WRS and AAT-WRS under different magnifications (1K, 2K, and 5K).

Fig. 3. DSC heat flow curves for native WRS and AAT-WRS.

Fig. 4. XRD patterns of native WRS and AAT-WRS

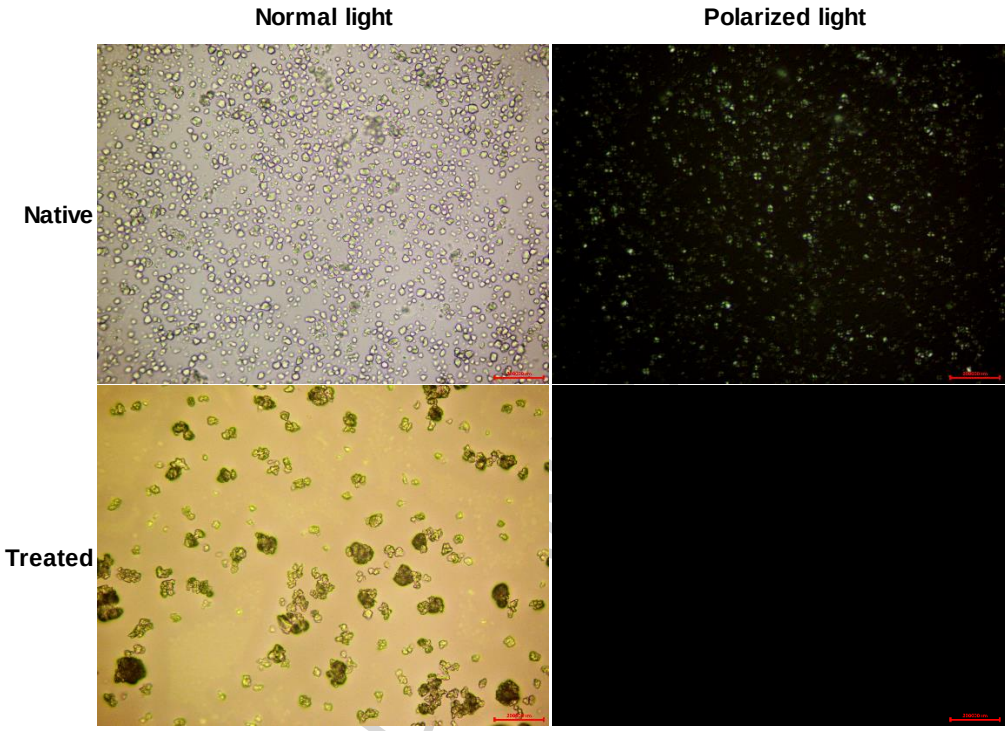
Fig. 5. FTIR spectra for native WRS and AAT-WRS.

Fig. 6. TGA derivative weight curves for native WRS and AAT-WRS.

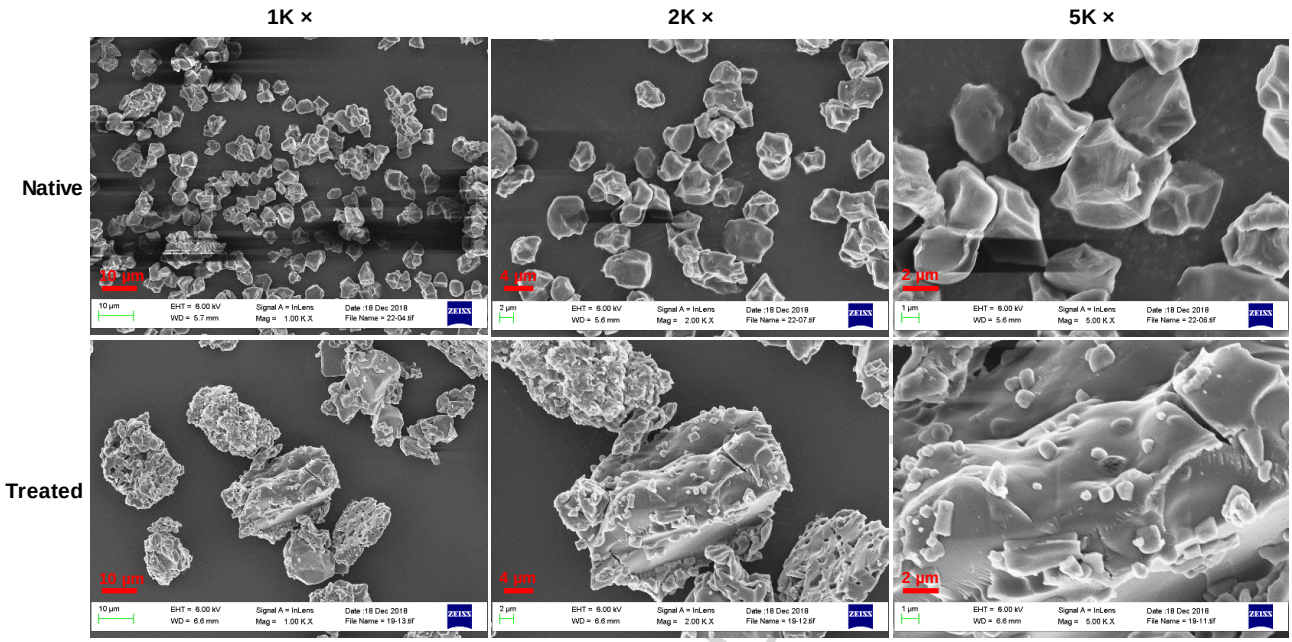
Fig. 7. Steady shear viscosity (η) as a function of shear rate ($\dot{\gamma}$) for ungelatinized native WRS, gelatinized native WRS, and AAT-WRS suspensions, all at 5% (w/w) concentration in water. The straight lines are the fitting curves.

Fig. 8. Storage modulus (G'), loss modulus (G''), and loss tangent (tan δ) as a functions of frequency (ω) at a fixed temperature of 25 °C and as a function of temperature at a fixed frequency of 5 rad/s (b) for gelatinized native WRS and AAT-WRS suspensions, both at 5% (w/w) concentration in water. The strain was kept at 1.5%.

Figures



International Journal of Biological Macromolecules – Chen et al. – Fig. 1



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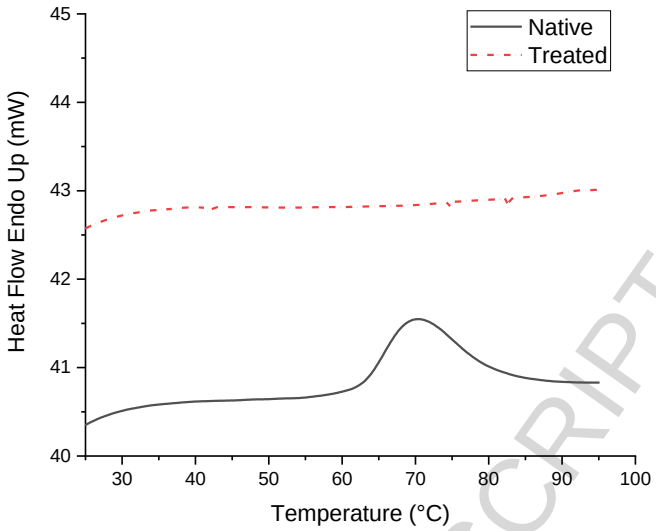

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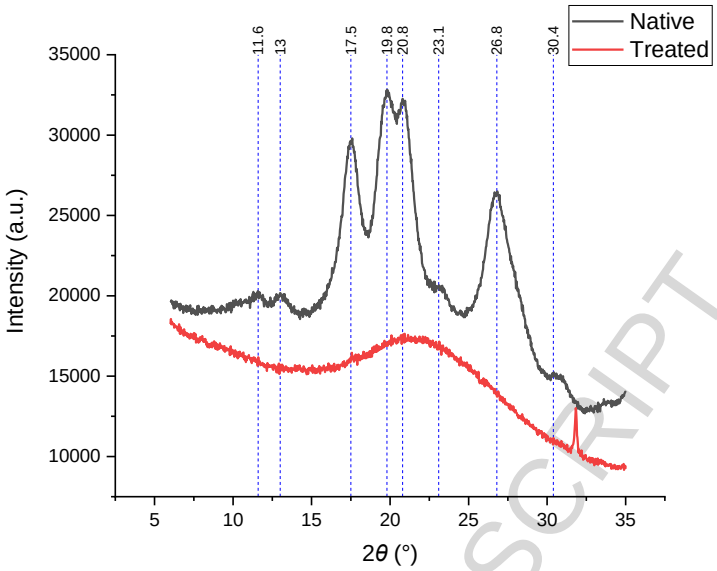

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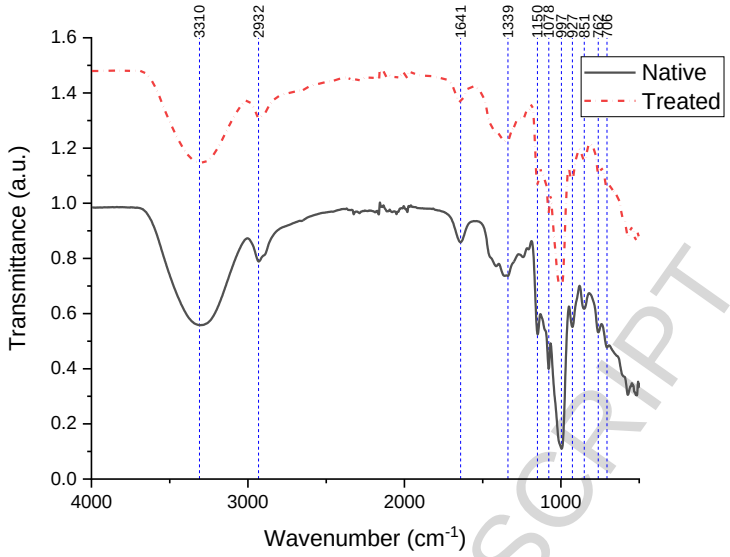
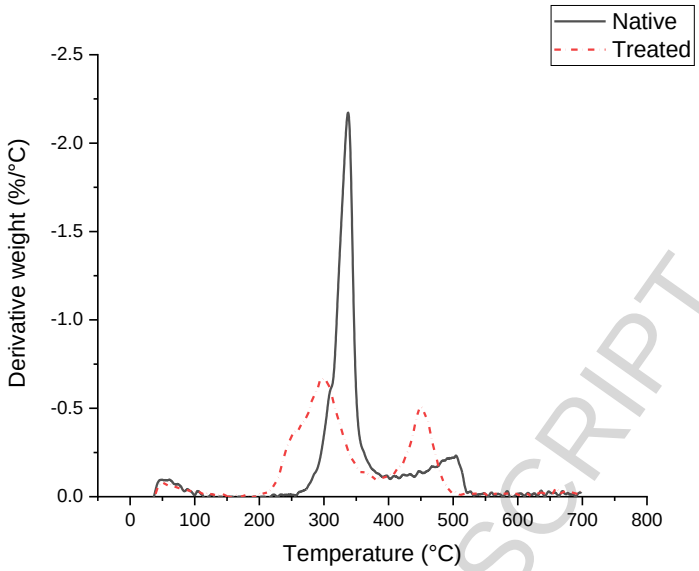
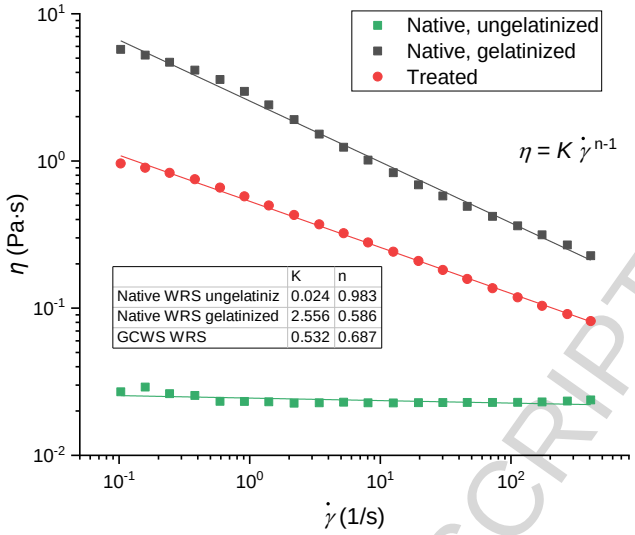


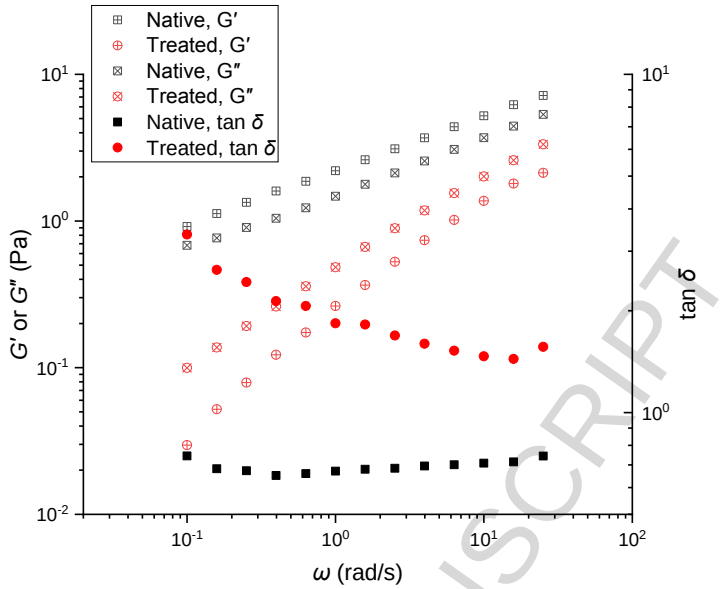
International Journal of Biological Macromolecules – Chen et al. – Fig. 5



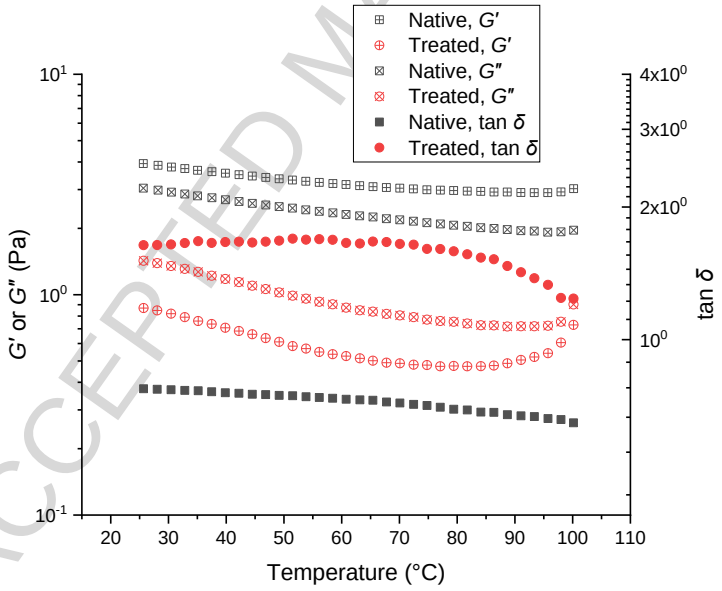
International Journal of Biological Macromolecules – Chen et al. – Fig. 6



International Journal of Biological Macromolecules – Chen et al. – Fig. 7



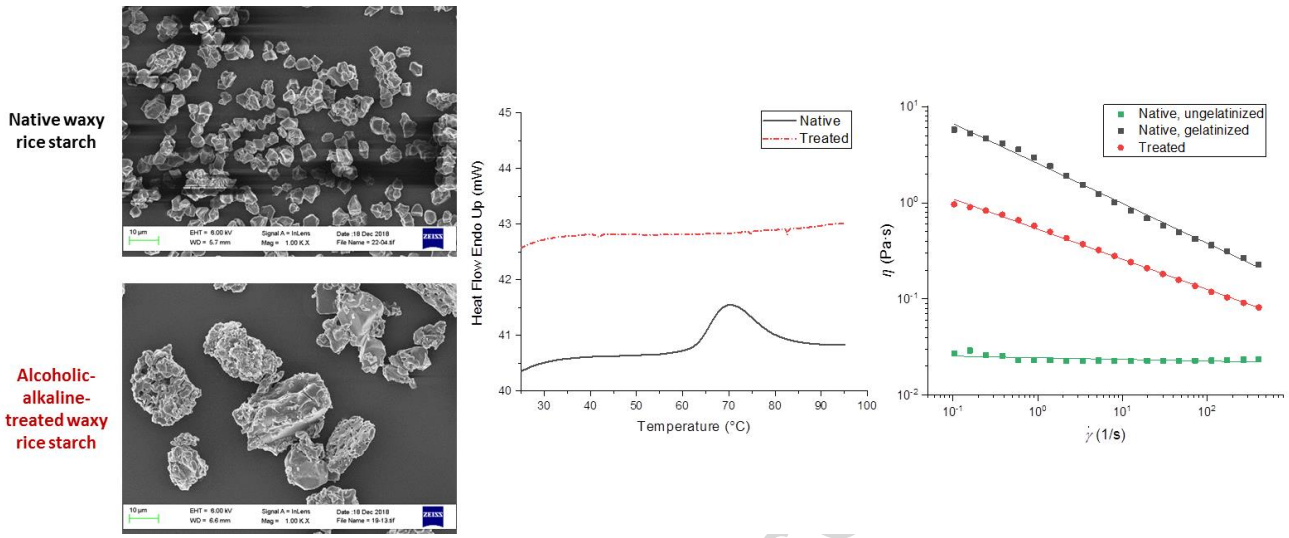
(a)



(b)

International Journal of Biological Macromolecules – Chen et al. – Fig. 8

Graphical abstract



Highlights

- ✓ Alcoholic-alkaline-treated waxy rice starch (AAT-WRS) was in the form of particles
- ✓ AAT-WRS was completely amorphous
- ✓ AAT-WRS presented reduced thermal decomposition temperature
- ✓ AAT-WRS suspensions exhibited less-rigid gel structure than gelatinised WRS
- ✓ The rigidity of AAT-WRS suspensions decreased with higher temperature